Protocol for Neutralizing Antibody Assay Reagent Bridging Studies (April 2020)

I. INTRODUCTION

The Duke Neutralizing Antibody Assay Laboratory is responsible for assessing vaccine elicited neutralizing antibody responses in clinical trials of candidate HIV-1 vaccines. Bridging studies must be performed on reagents, when new lot numbers of reagents or preparations of cells or viruses are available, to ensure the integrity of the reagents and the validity of the assay. All current and new reagents for bridging studies will be evaluated using the neutralizing antibody assay in TZM-bl cells.

II. DEFINITIONS

DEAE: Diethylaminoethyl

DPBS: Dulbecco's Phosphate Buffered Saline

EDTA: Ethylenediaminetetraacetic Acid

FBS: Fetal Bovine Serum

IBMT: Fraunhofer Institut fur Biomedizinische Technik

IMC: Infectious Molecular Clone

NIH: National Institutes of Health

PI: Principal Investigator

QBI: Quality Biological Inc.

RLU: Relative Light Units

TCID: Tissue Culture Infectious Dose

II. Specimens

Control reagents, Fetal Bovine Serum (FBS), Env-pseudotyped viruses, Env.Infectious Molecular Clone (IMC) viruses, cells (TZM-bl, 293T/17, 293S GnTI-) listed in various protocols.

III. REAGENTS AND MATERIALS

Recommended vendors are listed. Unless otherwise specified, products of equal or better quality may be used when necessary.

Control Reagents (sCD4, IgG1b12, 2F5, 4E10, TriMAb, 2G12, CH01-31)
Polymun, Progenics, Catalent, QBI

Fetal Bovine Serum
Nucleus Biologics

TZM-bl Cells
NIH AIDS Reagent Program

293T/17 Cells
American Type Culture Collection

293S GnTI- Cells
American Type Culture Collection

Env-pseudotyped viruses
Duke Central Reference Laboratory
Fraunhofer Institute for Biomedizinische Technik (IBMT)

IMC Viruses
Duke Central Reference Laboratory
Fraunhofer Institute for Biomedizinische Technik IBMT (IBMT)

Growth Medium
Invitrogen, Sigma-Aldrich, Nucleus Biologics

DEAE-Dextran, hydrochloride, avg. Mol. Wt. 500,000
Sigma

Trypsin-EDTA (0.25% trypsin, 1mM EDTA)
Sigma

Dulbecco's Phosphate Buffered Saline (DPBS), Sterile
Invitrogen

Trypan Blue (0.4%)
Invitrogen

Britelite Plus Reporter Gene Assay System
PerkinElmer Life and Analytical Sciences

Viviren Live Cell Substrate
Promega

Microliter pipettor tips
Eppendorf, RAININ, Biohit

Disposable Pipettes, sterile, individually wrapped (1 ml, 5 ml, 10 ml, 25 ml, 50 ml)
Costar / VWR

Flat-bottom culture plates, 96-well, low evaporation, sterile
Corning

Flat-bottom black solid plates, 96-well, Costar brand
Perkin Elmer

Reagent reservoirs, 25 ml, 50 ml, 100 ml
Denville Scientific

Culture flasks with vented caps, sterile (T-75)
Denville Scientific

Equipment:

Luminometer
Perkin Elmer Life Science

Biological Safety Cabinet
The Baker Company, Inc.

Incubator
Panasonic

Pipettor
Sartorius, RAININ, Eppendorf, Drummond

Light Microscope
Olympus, ThermoFisher Scientific

Centrifuge and Microcentrifuge
Jouan, Eppendorf

Hemacytometer
INCYTO

Water Bath
Precision Scientific

Laboratory Refrigerator / -20°C Freezer
Sci-Cool, LABRepCo

Low Temperature Freezer
Revco/Harris, ThermoFisher Scientific

Liquid Nitrogen Freezer Tank
MVE, Inc.

IV. PROTOCOL

Control Reagents

NOTE 1: Control Reagents (e.g., sCD4, IgG1b12, 2F5, 4E10, TriMab, 2G12 and CH01-31). Control reagents are bridged only in TZM-bl cells. As per the Principal Investigator (PI), it is sufficient to perform the bridging of control reagents in one cell line.

1. A bridging study should be performed each time a new lot of a control reagent is received from the manufacturer.

NOTE 2: A bridging study should be done on a reagent of the same lot if an extended period of time has passed since the initial bridging was performed.

2. Run a parallel test of a current lot (record the lot number or receipt date in Appendix A) with the new lot (record in Appendix A) of the control antibody using the neutralizing antibody assay in TZM-bl cells.

3. At least three viruses should be used to bridge Control Reagents. Perform the assay with HIV-1 SF162.LS/293T/17, HIV-1 QH0692.42/293T/17 and any other viruses assigned by the PI.

    a. For routine set up, use a 0.5 mg/ml stock solution of the assay control. Start at a 1:20 dilution and do 3-fold dilutions (final starting concentration = 25 µg/ml). The starting concentration of controls will vary as indicated by the neutralizing antibody sensitivity of the virus.

4. Pass/Fail Criteria

    a. Each assayed plate must pass Pass/Fail Criteria set for the neutralization assay. In addition the following criteria must also be met.

    b. Pass: Test results for at least two/two assayed reagents agree within 3-fold between the two sets of data. The mean Relative Light Units (RLU) of the virus control wells must be at least 10x the background for the plate.

    c. Fail: Test results for at least one/two assayed reagents are > 3-fold between the two sets of data. The mean RLU of the virus control wells are less than 10x the background for the plate. The test will be repeated as necessary.

Virus Preparation

1. A bridging study should be performed each time a new batch of an existing virus is prepared and after the TCID is completed. IMC(LucR) viruses and pseudoviruses will be bridged in appropriate cell lines sensitive to each particular virus.

NOTE 3: The new batch of virus should be run against a previous batch of the virus that has already passed the bridging criteria (see below).

NOTE 4: A phenotyping assay should be performed each time a new virus (i.e., no other batches available for comparison) is made. It will be up to the discretion of the PI (or the designee) to determine if unique viruses are to be phenotyped.

2. Perform the bridging assay with a previously bridged batch of virus (record harvest date in Appendix B) along with both harvest dates of the new virus (record in Appendix B) at the dilution indicated in the virus database.

3. Assay the viruses against any five out of the following control reagents: sCD4, 4E10, HIVIG-C, CH01, CH31, CH01-31, VRC01 and TriMAb. The PI may also assign alternate control reagents to use in the bridging/phenotyping assay. The starting concentration of controls may vary as indicated by the neutralizing antibody sensitivity of the virus.

4. Start the assay at a 1:20 dilution and do 3-fold dilutions.

5. Pass/Fail Criteria

    a. Each assayed plate must pass Pass/Fail Criteria set for the neutralization assay. In addition the following criteria must also be met.

    b. Pass: Test results for at least four/five assayed reagents agree within 3-fold between the two sets of data. The mean Relative Light Units (RLU) of the virus control wells must be at least 10x the background for the plate.

    c. Fail: Test results for at least two/five assayed reagents are > 3-fold between the two sets of data. The mean RLU of the virus control wells are less than 10x the background for the plate. The test will be repeated as necessary.

NOTE 5: If the new batch of virus fails the repeated bridging assay, the new batch of virus will be discard.

NOTE 6: It will be upon the discretion of the Lab Manager (or designee) to assign a virus, that does not pass the 10X cell control criteria, but showing the correct neutralization profile, for research purposes only.

NOTE 7: Sometimes new batches of viruses are made in other labs and shipped to Duke. If the other lab bridges the virus and the virus passes, then it will be considered bridged and ready to be used in neutralization assays.

293T/17 and 293S/GnTI- Cell Integrity

1. Each time a new batch of 293T/17 cells is thawed, and before discarding the old cells, a virus should be grown in parallel using the old cells and the new cells and the viruses should be bridged as described in “Virus Preparation” above. The yield of virus grown in the new batch of cells should not be lower than 3-fold compared to the virus grown in the old batch of cells.

TZM-bl Cell Integrity

1. A bridging study should be performed each time a new aliquot of cells is passed into a culture from liquid nitrogen storage.

2. Perform the neutralization assay with the current culture of TZM-bl cells and the newly established culture of TZM-bl cells (record in Appendix C).

3. Assay the cells with HIV- SF162.LS/293T/17 and HIV-1 QH0692.42/293T/17 (or alternate viruses assigned by the PI) when testing TZM-bl cells.

4. Assay the viruses against any five of the following control reagents: sCD4, 4E10, HIVIG-C, CH01, CH01-31 and TriMAb or use alternate control reagents assigned by the PI.

    a. The starting concentration of controls will vary as indicated by the neutralizing antibody sensitivity of the virus.

    b. Consult with the PI in order to proceed with the appropriate antibody concentration with that will yield a full concentration curve at 50% neutralization.

5. Pass/Fail Criteria

    a. Each assayed plate must pass Pass/Fail Criteria set for the neutralization assay. In addition, the following criteria must also be met.

    b. Pass: Test results for at least four/five assayed reagents agree within 3-fold between the two sets of data. The mean RLU of the virus control wells must be at least 10x the background for the plate.

    c. Fail: Test results for at least two/five assayed reagents are > 3-fold between the two sets of data. The mean RLU of the virus control wells are less than 10x the background for the plate. The test will be repeated as necessary.

NOTE 8: If cells fail repeated bridging assays, a new batch will be retrieved from liquid nitrogen storage.

Fetal Bovine Serum

1. Perform a bridging study each time a new lot number of FBS is received from the manufacturer.

2. Perform the neutralization assay with the current and the new lot numbers of FBS.

NOTE 9: Growth medium should be prepared using the new lot number of FBS and a flask of cells kept in culture in the new growth medium for at least two passages prior to performing the test. Perform the neutralizing antibody assay in parallel using cells kept in growth medium prepared with the old lot number of FBS and using cells cultured in growth medium with the new lot of FBS (record in Appendix D).

3. Perform the bridging assay using pseudoviruses in TZM-bl cells.

4. Assay viruses against any five out of the following list of control reagents: sCD4, 4E10, HIVIG-C, CH01, CH31 CH01-31 and TriMAb or use alternate control reagents assigned by the PI.

5. Pass/Fail Criteria

    a. Each assayed plate must pass Pass/Fail Criteria established for the neutralization assay. In addition, the following criteria must also be met.

b. Pass: Test results for at least four/five assayed control reagents (or two/two reagents in Control Reagent Bridging testing) agree within 3-fold between the two sets of data. The mean RLU of the virus control wells must be at least 10x the mean RLU value of the cell control wells of the plate.

c. Fail: Test results for at least two/five assayed reagents (or one/two reagents in Control Reagent Bridging testing) are > 3-fold between the two sets of data. The mean RLU values of the virus control wells are less than 10X than the mean RLU values of the cell control wells of the plate. The test will be repeated as necessary. If a failed reagent cannot pass the bridging test, the reagents should not be used.

Procedure for Recording and Reviewing Results

1. The technician should record the bridging results on the appropriate Bridging Testing sheet.

2. The technician performing the bridging assay should sign the Bridging Testing sheet(s).

3. The technician should submit the Bridging Testing sheet(s), along with the raw data, to the PI (or designee) for review and signature. The reviewer should indicate on the sheet(s) whether the reagent/virus used in the bridging test has passed or failed the established criteria.

4. The Bridging Testing sheet(s), along with the appropriate raw data and communication material, if applicable, should be filed within the Bridging Studies notebook. Additionally, bridging results (and assay number) should be entered in the Virus Database.

V. REFERENCES

1. TZM-bl assay protocols

VI. APPENDICES

Appendix A: Control Reagent Bridging Testing Record

Neutralizing Antibody Assay **Control Reagent Parallel Testing**

Date:	Tech:	Virus:
Current Control:	New Control:	Virus Date:
Current Control Lot Number:	New Control Lot Number:	Virus ID:
Current Control Date Received:	New Control Date Received:	Experiment #:
Current Control Manufacturer:	New Control Manufacturer:	Parallel Testing Passed[1]:
ID50 in TZM-bl Cells (µg/ml)	ID50 in TZM-bl Cells (µg/ml)	Date in Use:

Signature: _____ Date: _____

Reviewed: _____ Date: _____

Date:	Tech:	Virus:
Current Control:	New Control:	Virus Date:
Current Control Lot Number:	New Control Lot Number:	Virus ID:
Current Control Date Received:	New Control Date Received:	Experiment #:
Current Control Manufacturer:	New Control Manufacturer:	Parallel Testing Passed[1]:
ID50 in TZM-bl Cells (µg/ml)	ID50 in TZM-bl Cells (µg/ml)	Date in Use:

Signature: _____ Date: _____

Reviewed: _____ Date: _____

Appendix B: Pseudovirus Preparation Testing Record

Neutralizing Antibody Assay Pseudovirus Preparation Parallel Testing

Date:					Tech:					
Virus:					Experiment #:					
Current Virus Preparation Date:					New Virus Preparation Date:					
Virus ID:					Virus ID:					
TCID:					TCID:					
ID50 in TZM-bl Cells (µg/ml)					ID50 in TZM-bl Cells (µg/ml)					
sCD4	IgG1b12	2F5	4E10	2G12	sCD4	IgG1b12	2F5	4E10	2G12	Parallell Testing Passed[1]

Signature: _____ Date: _____
Reviewed: _____ Date: _____

Date:					Tech:					
Virus:					Experiment #:					
Current Virus Preparation Date:					New Virus Preparation Date:					
Virus ID:					Virus ID:					
TCID:					TCID:					
ID50 in TZM-bl Cells (µg/ml)					ID50 in TZM-bl Cells (µg/ml)					
sCD4	IgG1b12	2F5	4E10	TriMab	sCD4	IgG1b12	2F5	4E10	TriMab	Parallell Testing Passed[1]

Signature: _____ Date: _____
Reviewed: _____ Date: _____

Appendix C: TZM-bl Cell Integrity Post Thaw Bridging Testing Record

Neutralizing Antibody Assay TZM-bl Cell Integrity Post Thaw Testing

Date:					Tech:						
Current Culture:					New Culture:					Virus:	
Passage Number:					Passage Number:					Virus Date:	
Thaw Date:					Thaw Date:					Virus ID:	
										Experiment #:	
ID50 in TZM-bl Cells (µg/ml)					ID50 in TZM-bl Cells (µg/ml)						
sCD4	IgG1b12	2F5	4E 10	TriMab	sCD4	IgG1b12	2F5	4E 10	TriMab	Parallell Testing Passed[1]	Date new culture in use

Signature: _____ Date: _____

Reviewed: _____ Date: _____

Date:					Tech:						
Current Culture:					New Culture:					Virus:	
Passage Number:					Passage Number:					Virus Date:	
Thaw Date:					Thaw Date:					Virus ID:	
										Experiment #:	
ID50 in TZM-bl Cells (µg/ml)					ID50 in TZM-bl Cells (µg/ml)						
sCD4	IgG1b12	2F5	4E 10	TriMab	sCD4	IgG1b12	2F5	4E 10	TriMab	Parallell Testing Passed[1]	Date new culture in use

Signature: _____ Date: _____

Reviewed: _____ Date: _____

Appendix D: Fetal Bovine Serum (FBS) Bridging Testing Record

Neutralizing Antibody Assay Fetal Bovine Serum (FBS) Lot to Lot Parallel Testing

Date:					Tech:						
Current Lot:					New Lot:					Virus:	
Lot Number:					Lot Number:					Virus Date:	
Expiration Date:					Expiration Date:					Virus ID:	
					Received Date:					Experiment #:	
ID50 in TZM-bl Cells (µg/ml)					ID50 in TZM-bl Cells (µg/ml)						
sCD4	IgG1b12	2F5	4E 10	TriMab	sCD4	IgG1b12	2F5	4E 10	TriMab	Parallell Testing Passed[1]	Date new culture in use

Signature: _____ Date: _____
Reviewed: _____ Date: _____

Date:					Tech:						
Current Lot:					New Lot:					Virus:	
Lot Number:					Lot Number:					Virus Date:	
Expiration Date:					Expiration Date:					Virus ID:	
					Received Date:					Experiment #:	
ID50 in TZM-bl Cells (µg/ml)					ID50 in TZM-bl Cells (µg/ml)						
sCD4	IgG1b12	2F5	4E 10	TriMab	sCD4	IgG1b12	2F5	4E 10	TriMab	Parallell Testing Passed[1]	Date new culture in use

Signature: _____ Date: _____
Reviewed: _____ Date: _____